

IN THE SPECIFICATION:

Please replace the second full paragraph of page 2, with the following paragraph:

The [Ehrlichia] Ehrlichia are obligate intracellular pathogens that infect circulating lymphocytes in mammalian hosts. *Ehrlichia canis* and *Ehrlichia chaffeensis* are members of the same sub-genus group that infect canines and humans and cause canine monocytic ehrlichiosis (CME) and human monocytic ehrlichiosis (HME), respectively. The canine disease is characterized by fever, lymphadenopathy, weight loss, and pancytopenia. In humans the disease is characterized by fever, headache, myalgia, and leukopenia. Early detection and treatment are important for treating both canine and human ehrlichiosis.

Please replace the third full paragraph of page 2 with the following paragraph:

Indirect immunofluorescence assays (IFA) and enzyme-linked immunosorbent assays (ELISA) are frequently used as aids in the diagnosis of these diseases. These assays measure or otherwise detect the binding of [anti-Ehrlichia] anti-Ehrlichia antibodies from a patient's blood, plasma, or serum to infected cells, cell lysates, or purified [Ehrlichia] Ehrlichia proteins. However, currently known assays for detecting [anti-Ehrlichia] anti-Ehrlichia antibodies or fragments thereof are severely limited in usefulness because of sensitivity and specificity issues directly related to the impure nature of the [Ehrlichia] Ehrlichia antigen used in these tests. Highly purified reagents are needed to construct more accurate assays.

Please replace the forth full paragraph of page 3 with the following paragraph:

Still another embodiment of the invention provides a method of detecting the presence of antibodies to [Ehrlichia] Ehrlichia. The method comprises contacting one or

more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with a test sample suspected of comprising antibodies to [Ehrlichia] Ehrlichia, under conditions that allow polypeptide/antibody complexes to form. The polypeptide/antibody complexes are detected. The detection of polypeptide/antibody complexes is an indication that antibodies to [Ehrlichia] Ehrlichia are present in the test sample.

Please replace the first full paragraph of page 4 with the following paragraph:

Yet another embodiment of the invention provides a device containing one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, and instructions for use of the one or more polypeptides for the identification of an [Ehrlichia] Ehrlichia infection in a mammal.

Please replace the second full paragraph of page 4 with the following paragraph:

Still another embodiment of the invention provides an article of manufacture comprising packaging material and, contained within the packaging material, one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof. The packaging material comprises a label that indicates that the one or more polypeptides can be used for the identification of [Ehrlichia] Ehrlichia infection in a mammal.

Please replace the third full paragraph of page 4 with the following paragraph:

Even another embodiment of the invention provides a method of diagnosing an

[Ehrlichia] Ehrlichia infection in a mammal. The method comprises obtaining a biological sample from a mammal suspected of having an [Ehrlichia] Ehrlichia infection, and contacting one or more polypeptides selected from the group consisting of the polypeptides shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and variants thereof, with the biological sample under conditions that allow polypeptide/antibody complexes to form. Polypeptide/antibody complexes are detected, wherein the detection of polypeptide/antibody complexes is an indication that the mammal has an [Ehrlichia] Ehrlichia infection.

Please replace the first full paragraph of page 5 with the following paragraph:

The invention therefore provides highly purified polypeptides and antibodies for use in accurate assays for the detection of [Ehrlichia] Ehrlichia antibodies and antibody fragments.

Please replace the third full paragraph of page 7 with the following paragraph:

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, the amino acid positions which have been conserved between species can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions in which substitutions have been tolerated by natural selection indicate positions which are not critical for protein function. Thus, positions tolerating amino acid substitution may be modified while still maintaining specific binding activity of the polypeptide to [anti-Ehrlichia] anti-Ehrlichia antibodies or antibody fragments.

Please replace the first full paragraph of page 8 with the following paragraph:

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site-directed mutagenesis or alanine-scanning mutagenesis (the introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham *et al.*, *Science*, 244:1081-1085 (1989)). The resulting mutant molecules can then be tested for specific binding to [anti-Ehrlichia] anti-Ehrlichia antibodies or antibody fragments.

Please replace the first full paragraph of page 9 with the following paragraph:

Polypeptides of the invention specifically bind to an [anti-Ehrlichia] anti-Ehrlichia antibody. In this context “specifically binds” means that the polypeptide recognizes and binds to an [anti-Ehrlichia] anti-Ehrlichia antibody, but does not substantially recognize and bind other molecules in a test sample.

Please replace the second full paragraph of page 9 with the following paragraph:

Polypeptides of the invention comprise at least one epitope that is recognized by an [anti-Ehrlichia] anti-Ehrlichia antibody. An epitope is an antigenic determinant of a polypeptide. An epitope can be a linear, sequential epitope or a conformational epitope. Epitopes within a polypeptide of the invention can be identified by several methods. *See, e.g.*, U.S. Patent No. 4,554,101; Jameson & Wolf, *CABIOS* 4:181-186 (1988). For example, a polypeptide of the invention can be isolated and screened. A series of short peptides, which together span the entire polypeptide sequence, can be prepared by proteolytic cleavage. By starting with, for example, 20-mer polypeptide fragments, each fragment can be tested for the presence of epitopes recognized in, for example, an enzyme-linked immunosorbent assay (ELISA). In an ELISA assay a polypeptide, such as a 20-mer polypeptide fragment, is attached to a solid support, such as the wells of a

plastic multi-well plate. A population of antibodies are labeled, added to the solid support and allowed to bind to the unlabeled antigen, under conditions where non-specific adsorption is blocked, and any unbound antibody and other proteins are washed away. Antibody binding is detected by, for example, a reaction that converts a colorless indicator reagent into a colored reaction product. Progressively smaller and overlapping fragments can then be tested from an identified 20-mer to map the epitope of interest.

Please replace the first full paragraph of page 10 with the following paragraph:

Preferably, a polypeptide of the invention is synthesized using conventional peptide synthesizers, which are well known in the art. A polypeptide of the invention can also be produced recombinantly. A polynucleotide encoding an [Ehrlichia] Ehrlichia polypeptide can be introduced into an expression vector that can be expressed in a suitable expression system using techniques well known in the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are available in the art and any such expression system can be used. Optionally, a polynucleotide encoding an [Ehrlichia] Ehrlichia polypeptide can be translated in a cell-free translation system.

Please replace the second full paragraph of page 10 with the following paragraph:

If desired, an [Ehrlichia] Ehrlichia polypeptide can be produced as a fusion protein, which can also contain other amino acid sequences, such as amino acid linkers or signal sequences, as well as ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. More than one [Ehrlichia] Ehrlichia polypeptide can be present in a fusion protein. If desired, various combinations of [Ehrlichia] Ehrlichia polypeptides from different [ehrlichia] Ehrlichia strains or isolates can be included in a fusion protein.

Please replace the third full paragraph of page 10 with the following paragraph:

A polypeptide of the invention can be synthesized such that it comprises several repeated [Ehrlichia] Ehrlichia polypeptides. This is a multimeric polypeptide. These repeated polypeptides can comprise one specific polypeptide, *e.g.* the polypeptide shown in SEQ ID NO:1, repeated 2 or more times. Alternatively, the repeated polypeptides can comprise one or more copies of a specific [Ehrlichia] Ehrlichia polypeptide along with one or more copies of another different [Ehrlichia] Ehrlichia polypeptide. A polypeptide of the invention can be combined or synthesized with one or more polypeptides, fragments of polypeptides, or full-length polypeptides. Preferably the one or more polypeptides are other polypeptides of the invention or other [Ehrlichia] Ehrlichia proteins.

Please replace the third full paragraph of page 11 with the following paragraph:

Various strains and isolates of *Ehrlichia canis* and *Ehrlichia chaffeensis* occur, and polypeptides of any of these strains and isolates can be used in the present invention. Nucleic acid and amino acid sequences of [Ehrlichia] Ehrlichia genes and polypeptides are known in the art. For example, several sequences of the *E. chaffeensis* OMP gene family and several sequences of the *E. canis* P30 gene family are disclosed in WO 99/13720.

Please replace the first full paragraph of page 12 with the following paragraph:

The methods comprise contacting a polypeptide of the invention with a test sample under conditions that allow a polypeptide/antibody complex to form. The formation of a complex between the polypeptide and [anti-Ehrlichia] anti-Ehrlichia antibodies in the sample is detected. In one embodiment of the invention, the

polypeptide/antibody complex is detected when an indicator reagent, such as an enzyme, which is bound to the antibody, catalyzes a detectable reaction. Optionally, an indicator reagent comprising a signal generating compound can be applied to the polypeptide/antibody complex under conditions that allow formation of a polypeptide/antibody/indicator complex. The polypeptide/antibody/indicator complex is detected. Optionally, the polypeptide or antibody can be labeled with an indicator reagent prior to the formation of a polypeptide/antibody complex. The method can optionally comprise a positive or negative control.

Please replace the first full paragraph of page 13 with the following paragraph:

Polypeptides of the invention can be used to detect [anti-Ehrlichia] anti-Ehrlichia antibodies or antibody fragments in assays including, but not limited to enzyme linked immunosorbent assay (ELISA), western blot, IFA, radioimmunoassay (RIA), hemagglutination (HA), and fluorescence polarization immunoassay (FPIA). A preferred assay of the invention is the reversible flow chromatographic binding assay, for example a SNAP® assay. See U.S. Pat. No. 5,726,010.

Please replace the second full paragraph of page 13 with the following paragraph:

In one type of assay format, one or more polypeptides can be coated on a solid phase or substrate. A test sample suspected of containing [anti-Ehrlichia] anti-Ehrlichia antibodies is incubated with an indicator reagent comprising a signal generating compound conjugated to an antibody specific for [Ehrlichia] Ehrlichia for a time and under conditions sufficient to form antigen/antibody complexes of either antibodies of the test sample to the polypeptides of the solid phase or the indicator reagent compound conjugated to an antibody specific for [Ehrlichia] Ehrlichia to the polypeptides of the

solid phase. The reduction in binding of the indicator reagent conjugated to an [anti-Ehrlichia] anti-Ehrlichia antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative [Ehrlichia] Ehrlichia test sample indicates the presence of [anti-Ehrlichia] anti-Ehrlichia antibody in the test sample. This type of assay can quantitate the amount of [anti-Ehrlichia] anti-Ehrlichia antibodies in a test sample.

Please replace the first full paragraph of page 14 with the following paragraph:

In another type of assay format, one or more polypeptides of the invention are coated onto a support or substrate. A polypeptide of the invention is conjugated to an indicator reagent and added to a test sample. This mixture is applied to the support or substrate. If [Ehrlichia] Ehrlichia antibodies are present in the test sample they will bind the polypeptide conjugated to an indicator reagent and to the polypeptide immobilized on the support. The polypeptide/antibody/indicator complex can then be detected. This type of assay can quantitate the amount of [anti-Ehrlichia] anti-Ehrlichia antibodies in a test sample.

Please replace the second full paragraph of page 15 with the following paragraph:

The methods of the invention can also indicate the amount or quantity of [anti-Ehrlichia] anti-Ehrlichia antibodies in a test sample. With many indicator reagents, such as enzymes, the amount of antibody present is proportional to the signal generated. Depending upon the type of test sample, it can be diluted with a suitable buffer reagent, concentrated, or contacted with a solid phase without any manipulation. For example, it usually is preferred to test serum or plasma samples which previously have been diluted,

or concentrate specimens such as urine, in order to determine the presence and/or amount of antibody present.

Please replace the third full paragraph of page 15 with the following paragraph:

The invention further comprises assay kits for detecting [anti-Ehrlichia] anti-Ehrlichia antibodies in a sample. A kit comprises one or more polypeptides of the invention and means for determining binding of the polypeptide to [Ehrlichia] Ehrlichia antibodies in the sample. A kit can comprise a device containing one or more polypeptides of the invention and instructions for use of the one or more polypeptides for the identification of an [Ehrlichia] Ehrlichia infection in a mammal. The kit can also comprise packaging material comprising a label that indicates that the one or more polypeptides of the kit can be used for the identification of [Ehrlichia] Ehrlichia infection. Other components such as buffers, controls, and the like, known to those of ordinary skill in art, may be included in such test kits. The polypeptides, assays, and kits of the invention are useful, for example, in the diagnosis of individual cases of [Ehrlichia] Ehrlichia infection in a patient, as well as epidemiological studies of [Ehrlichia] Ehrlichia outbreaks.

Please replace the first full paragraph of page 16 with the following paragraph:

Polypeptides and assays of the invention can be combined with other polypeptides or assays to detect the presence of [Ehrlichia] Ehrlichia along with other organisms. For example, polypeptides and assays of the invention can be combined with reagents that detect heartworm and/or *Borrelia burgdorferi*.

Please replace the third full paragraph of page 16 with the following paragraph:

The antibodies or fragments thereof can be employed in assay systems, such as a reversible flow chromatographic binding assay, enzyme linked immunosorbent assay, western blot assay, or indirect immunofluorescence assay, to determine the presence, if any, of [Ehrlichia] *Ehrlichia* polypeptides in a test sample. In addition, these antibodies, in particular monoclonal antibodies, can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific [Ehrlichia] *Ehrlichia* proteins from, for example, cell cultures or blood serum, such as to purify recombinant and native [Ehrlichia] *Ehrlichia* antigens and proteins. The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

Please replace the fourth full paragraph of page 16 with the following paragraph:

Monoclonal antibodies directed against [Ehrlichia] *Ehrlichia* epitopes can be produced by one skilled in the art. The general methodology for producing such antibodies is well-known and has been described in, for example, Kohler and Milstein, Nature 256:494 (1975) and reviewed in J. G. R. Hurrel, ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press Inc., Boca Raton, Fla. (1982), as well as that taught by L. T. Mimms *et al.*, Virology 176:604-619 (1990). Immortal antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus.

Please replace the second full paragraph of page 18 with the following paragraph:

The synthetic peptide SNAP® assay and native antigen SNAP® assay comprised an assay system similar to that described in U.S. Pat. No. 5,726,010. Briefly, a test

sample is applied to a reverse flow chromatographic binding assay device and allowed to flow along and saturate a flow matrix. This facilitates sequential complex formation. That is, an [Ehrlichia] *Ehrlichia* antibody in the test sample binds first to a non-immobilized labeled specific binding reagent. In the case of the synthetic peptide SNAP® assay the non-immobilized labeled specific binding reagent is a polypeptide of the invention conjugated to horseradish peroxidase. For the native antigen SNAP® assay the reagent comprises partially purified native antigens. This complex binds to an immobilized analyte capture reagent. For the synthetic peptide SNAP® assay the immobilized analyte capture reagent is one or more polypeptides of the invention conjugated to bovine serum albumin. For the native antigen SNAP® assay the capture reagent is partially purified native antigens. An absorbent reservoir is contacted with the saturated flow matrix, thereby reversing the fluid flow. Detector and wash solution is delivered to the flow matrix. The liquid reagents remove unbound sample and unbound labeled specific binding reagent and facilitate detection of analyte complexes at the location of the of the immobilized analyte capture reagent. The substrate used in these experiments was 3,3',5,5' tetramethylbenzidine (TMB).